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STUDIES OF THE ANTIGENICITY OF HUMAN MALIGNANT MELANOMA FOR RABBITS

FINDINGS IN BLOOD SERUM OF ANTIBODY RESPONSE OF RABBITS FOLLOWING IMMUNIZATION PROCEDURES WITH EXTRACTS FROM MALIGNANT MELANOMAS AND CONTROL MATERIALS*

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Certain findings have been reported suggesting that materials in tissues from malignant melanomas of human beings can stimulate antibody formation. Hiramoto and Pressman (1) demonstrated by the fluorescent-tagged-antibody technic staining of sections of tumor tissue with antibody produced by injecting rabbits with tissue from human malignant melanoma. However, these authors found that other cutaneous tissues also showed affinity for the antibody-fluorescein conjugates. More recently, Blakemore and McKenna (2) demonstrated, by the technic of tanned-red-cell hemagglutination, appearance of antibodies in the serum of patients with malignant melanoma and a rise in titer of antibodies when they were injected with extracts of tissue from malignant melanomas. A number of other clinical and experimental observations (3–8) also suggest that materials in tissue from malignant melanomas can act as antigens.

Using the fluorescent antibody technic we too studied serologic effects in rabbits injected with malignant melanoma tissue and other control substances.

MATERIALS AND METHODS

1. Preparation of antiserums and antigens.—Tissue† from malignant melanomas, skin, gamma globulin and albumin from human beings and ovalbumin were used in standard immunization technic in the attempt to provoke antibodies.

Five tumor specimens, three melanotic and two amelanotic, were obtained. Surgical or post-mortem specimens of malignant melanoma were trimmed of fat, weighed and washed with normal saline until visibly free of blood. The tumor tissue was wrapped in aluminum foil and dropped into liquid nitrogen for about one minute. After such rapid freezing, the specimens were pulverized by the method of Wilhelmj *et al* (9). The material was stored in small aliquots at −20° C until ready for use. The tumor tissue was injected into a total of fifteen rabbits (8 albinos, 7 piebald) of an average weight of 2.5 kg. Surgical or autopsy specimens of normal human skin were trimmed of subcutaneous fat and processed in the same manner as the human malignant melanoma specimens. Human 7S gamma globulin was prepared from serum (10). Human albumin and ovalbumin were purchased‡. Each rabbit was bled prior to immunization, at monthly intervals thereafter and finally one week after the last injection. The details of the immunization procedures are listed in Table I.

2. Preparation of fluorescein-labeled conjugates.—The 7S gamma globulin portion of the following serums was isolated and labeled with fluorescein isothiocyanate (10):

1. Goat antirabbit gamma globulin serum§.
2. Pre-immunization serums of rabbits.
3. Post-immunization serums of rabbits.

Unconjugated aliquots of the same preparations were used for blocking.

3. Preparation of tissue powders for absorption studies.—Autopsy specimens of normal human kidney, spleen, lung, liver and brain were obtained. These were homogenized in a Virtis homogenizer at about 45,000 rpm for fifteen minutes. The suspension was then lyophilized and stored at −20° C until used.

Whole human blood was separated into plasma and cells. The plasma and blood cells were lyophilized separately and stored at −20° C.

Beef liver powder, beef spleen powder, lyophilized human gamma globulin and lyophilized human albumin were purchased‖.

4. Absorption of rabbit serums with tissue, plasma and blood cell powders.—For every ml of

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‡ Pentex Incorporated; Kankakee, Illinois.

§ Antibodies Incorporated, Davis, California.

‖ Pentex Incorporated; Kankakee, Illinois.

TABLE I

Schedules for immunization of rabbits

Antigen injected	Number of rabbits in each group	Major route of immunization	Antigen in Freund's adjuvant injected subcutaneously		Total period of injections
			−(no)	+(yes)	
Malignant melanoma tissue	10	Intravenous		+*	1 to 3½ months
	3	Intraperitoneal	−		1 to 3½ months
	2	Intravenous	−		3½ months
Human skin	2	Intravenous		+*	3½ months
Human 7S gamma globulin	10	Intravenous		+**	1 to 4½ months
	2	Intravenous	−		2 months
Human albumin	1	Intravenous	−		1 month
	2	Intravenous		+**	1 to 4½ months
Ovalbumin	3	Intravenous		+**	2 months
	3	Intraperitoneal	−		2 months
	3	Intravenous	−		2 months
Normal saline	4	Intravenous		+***	1 to 3 months

* 14 mg of antigen (wet weight) in 0.1 ml of saline mixed with 0.1 ml of complete Freund's adjuvant (Difco) was injected in the interscapular region once a week for two weeks. Booster injections containing 7 mg of antigen in 0.1 ml of normal saline were given intravenously three times a week.

** 10 mg of antigen in 0.1 ml of normal saline mixed with 0.1 ml of complete Freund's adjuvant was injected in the interscapular region once a week for two weeks. Booster injections of 1.5 mg of antigen were given intravenously three times a week for one week, followed by 2.0 mg intravenously three times a week thereafter.

*** 0.1 ml of normal saline mixed with 0.1 ml of complete Freund's adjuvant was injected in the interscapular area once a week for two weeks. Booster injections of 0.1 ml of normal saline were given intravenously three times a week.

serum, 200 mg of tissue powder was used. The powder was resuspended in saline (except in case of human plasma and blood cell powder) and centrifuged; the supernatant was discarded and the wet powder was added to the serum or conjugate to be absorbed. Three absorptions were made, each for one hour at room temperature. The mixture was constantly agitated during this time. After each absorption the suspension was centrifuged at 2500 rpm at 4° C for 20 minutes. The supernatant was removed and added to another 200 mg of wet powder. After the third absorption the serum or conjugate was used in the staining procedure.

5. Absorption of rabbit serums with human gamma globulin or human albumin.—For every ml of serum or conjugate 20 mg of powder was used. Two absorptions were made for one hour each at room temperature. After centrifugation the supernatant was removed and 20 mg of fresh powder was suspended in it. The mixture was kept at 37° C for one hour and then allowed to stand overnight at 4° C. After centrifugation the supernatant fluid was used in the staining procedure.

6. Processing of human tissue specimens for frozen tissue sections.—Immediately after excision the specimens were wrapped in aluminum foil and dropped into liquid nitrogen for one minute. Sections two to six microns thick were cut in a cryostat and placed on glass slides. The sections were then dried at room temperature for one hour, fixed in acetone for ten minutes and stored at −20° C until ready for staining by the fluorescent antibody technic. Alternate sections were stained with hematoxylin and eosin to correlate light microscopy findings with those of fluorescence microscopy. Where indicated, periodic acid-Schiff (PAS) with and without preliminary diastase digestion and reticulum (Wilder) stains were used.

7. Staining of tissue sections.—The indirect immunofluorescence technic was used (11) and when applicable the findings confirmed by the direct technic.

Some cryostat-cut sections were washed with buffered saline pH 7.5 for thirty minutes and then treated for thirty minutes with the rabbit serum to be tested. The sections were then washed 5

minutes × 4 with buffer following which fluorescein conjugated goat antirabbit gamma globulin was applied for thirty minutes. The tissue sections were again washed with buffer five minutes × 4. A coverslip was applied using Coon's buffered glycerol as the mounting medium (12). The following controls for specificity of staining were done:

1. A pre-immunization serum from each rabbit was used as a control for the post-immunization serum of the same rabbit.
2. Rabbit antiovalbumin serum and serum from rabbits injected with Freund's adjuvant and normal saline were used as controls.

Other sections were washed with Coon's buffered saline pH 7.5 for thirty minutes and then treated with fluorescein-conjugated rabbit antihuman malignant melanoma serum or fluorescein-conjugated rabbit antihuman skin serum.

The following controls for specificity of staining were done:

Unconjugated rabbit antihuman malignant melanoma serum or unconjugated rabbit antihuman skin serum was applied to the tissue sections for two hours. The sections were washed for ten minutes with two changes of buffer and then the corresponding fluorescein-conjugated rabbit antiserum was applied for thirty minutes.

After the final washing of the sections a coverslip was applied using Coon's buffered glycerol as mounting medium. The tissue sections were examined under a Leitz Ortholux fluorescence microscope using a 2 mm UG I for UV light excitation and a 2.5 mm Euphos barrier filter. Photographs were taken using Kodak high speed Ektachrome film for daylight. Exposure times varied from one to four minutes.

8. Passive cutaneous anaphylaxis (13).—Serums from rabbits injected with human malignant melanoma, human skin, human 7S gamma globulin and normal saline were injected intradermally in 0.1 ml amounts at different sites 1 cm apart into the backs of albino guinea pigs weighing about 250 grams. Two milliliter aliquots of Virtis homogenized emulsions (14 mg per ml) of human malignant melanoma or human skin were mixed with 0.5 ml of a 1% Evans blue solution in 0.15 M NaCl. Four hours after the intradermal injections of the serums, four guinea pigs were injected with the human skin and four guinea pigs were injected with the human malignant melanoma emulsions intravenously. The injected skin sites were observed for 45 minutes after the intravenous challenge. The guinea pigs were then killed, skinned and reactions measured on inner surface of skin. Observations were made for localization of dye.

RESULTS

The results of the staining reactions are listed in Tables II, III and IV.

Rabbits immunized with tissue from *human malignant melanoma, human skin* or *human 7S gamma globulin* developed serum antibodies which were shown by the immunofluorescence technic to react with: (a) stroma of malignant melanoma tissue (Fig. 1), (b) cytoplasm of plasma cells and/or large mononuclear cells within the tumor (Fig. 2), (c) stroma surrounding tubules in the human kidney (Fig. 3), (d) basement membrane of renal glomerular capillaries and Bowman's capsule (Fig. 4), (e) walls of some blood vessels, (f) pars papillaris of human skin, (g) scattered areas between dermal collagen bundles. No significant localization of antibodies in or on malignant melanoma tumor cells occurred (Fig. 1). The route or duration of injection did not significantly influence the results obtained. There was no apparent difference in the staining pattern after immunization of rabbits with human malignant melanoma, normal human skin, or human gamma globulin.

Rabbits immunized with *human albumin* developed serum antibodies which were shown by the immunofluorescence technic to stain those sites listed above except that they did not stain the cytoplasm of plasma cells and/or large mononuclear cells.

Details of results of staining after tissue powder absorptions are given in Tables II, III and IV. Absorption of the antiserums with human spleen abolished all specific green fluorescent staining seen on the tissue sections of human kidney, human malignant melanoma and human skin. Absorption of antiserums with either beef liver or beef spleen did not diminish the specific green fluorescent staining seen on the above tissue sections.

When the ovalbumin or complete Freund's adjuvant alone was injected into rabbits, the serums did not show staining of kidney, skin or malignant melanoma tissue except in the case of rabbit antiovalbumin serum where equivocal green fluorescence of the tumor cells occurred. Six rabbits out of twelve who were injected with human gamma globulin failed to develop antihuman gamma globulin antibody detectable by immunoelectrophoretic analysis or qualitative precipitin ring tests (14). These six serums did not show any of the above staining characteristics.

Post-immunization rabbit serums were separated into 7S and non-7S fractions by DEAE column chromatography. The 7S component of serums of rabbits injected with human skin and human malignant melanoma showed staining of

TABLE II

Staining reactions of human malignant melanoma

Serum	Cytoplasm of tumor cells	Cytoplasm of plasma and/or mononuclear cells	Stroma between tumor cells
Antihuman malignant melanoma, antihuman skin or antihuman gamma globulin serums	1+–N	4+	4+
Antihuman albumin serum	±–1+	N	2–3+
Antiovalbumin serum	±–N	N	N
Antihuman malignant melanoma serum absorbed with:			
1. Human kidney, spleen or lung	N	N	±–N
2. Human liver or human brain	N	N	1–2+
3. Human plasma, gamma globulin or human blood cells	N	N	3–4+
4. Human albumin, beef liver or beef spleen	N	4+	3–4+
Antihuman skin serum absorbed with:			
1. Human spleen	N	N	N
2. Human lung or human kidney	N	N	1–2+
3. Human liver	N	N	2–3+
4. Human brain, plasma, gamma globulin or blood cells	N	N	3–4+
5. Human albumin, beef liver or beef spleen	N	4+	3–4+
Antihuman gamma globulin serum absorbed with:			
1. Human kidney, spleen, lung, liver, brain or blood cells	N	N	N
2. Human gamma globulin	N	1+–N	1+
3. Human albumin or beef liver	N	4+	3–4+
Antihuman albumin serum absorbed with:			
1. Human kidney, lung, or spleen	N	N	N
2. Human brain, albumin, or blood cells	N	N	1–2+
3. Human plasma or human gamma globulin	1+	N	1+–N
4. Human liver, beef liver or beef spleen	±–1+	N	2–3+
Antihuman malignant melanoma serum after pretreatment with unlabeled homologous serum or unlabeled antihuman skin serum	N	N	±–N
Antihuman malignant melanoma serum after pretreatment with unlabeled normal rabbit serum	±–N	4+	4+
Normal Rabbit Serum	N	N	N

Code:

N—No specific fluorescence; ±—Equivocal green fluorescence; 1—Minimal green fluorescence; 2—Moderate green fluorescence; 3—Bright green fluorescence; 4—Brilliant green fluorescence.

stroma and mononuclear cells within malignant melanoma tissue sections using both the direct and indirect Coon's technics; whereas the non-7S component showed no staining by these methods. The 7S component of serums of rabbits injected with human gamma globulin did not show stromal staining; however, mononuclear cells were stained.

The staining of the cytoplasm of plasma cells and/or large mononuclear cells within the tumor could be eliminated by prior absorption of the serums with human gamma globulin. The staining of these cells was blocked by prior treatment of the tissue section with unconjugated rabbit antihuman gamma globulin.

Heating the serums at 56° C for thirty minutes did not affect the above reactions, nor did prior treatment of tissue sections with hyaluronidase.

The results of the passive cutaneous anaphy-

TABLE III

Staining reactions of human kidney

Serum	Glumerular basement membrane	Stroma around tubules	Walls of blood vessels
Antihuman malignant melanoma, antihuman skin, antihuman gamma globulin or antihuman albumin serums	3-4+	3-4+	3-4+
Antiovalbumin serum	N	N	N
Antihuman malignant melanoma serum absorbed with:			
1. Human spleen	N	N	N
2. Human kidney, lung or liver	N	N	3+
3. Human brain	N	2+	N
4. Human gamma globulin, albumin, blood cells, plasma, beef liver or beef spleen	3-4+	3-4+	3-4+
Antihuman skin serum absorbed with:			
1. Human spleen	N	N	N
2. Human kidney or human lung	N	N	1-2+
3. Human liver, plasma, gamma globulin, beef liver or beef spleen	3-4+	3-4+	3-4+
4. Human brain, blood cells or albumin	3-4+	3-4+	1-2+
Antihuman gamma globulin serum absorbed with:			
1. Human spleen or human brain	N	N	N
2. Human kidney, lung or liver	N	N	2-3+
3. Human blood cells	1-2+	1+	2-3+
4. Human gamma globulin, human albumin or beef liver	3-4+	3-4+	3-4+
Antihuman albumin serum absorbed with:			
1. Human spleen	N	N	N
2. Human kidney, lung, or liver	N	N	2-3+
3. Human brain	N	2-3+	N
4. Human plasma, gamma globulin or albumin	1-2+	N	2-3+
4. Human blood cells	3+	N	2+
6. Beef liver or beef spleen	3-4+	4+	3+
Antihuman malignant melanoma serum after pretreatment with unlabeled homologous serum or unlabeled antihuman skin serum	±-N	±-N	±-1+
Antihuman malignant melanoma serum after pretreatment with unlabeled normal rabbit serum	4+	4+	4+
Normal rabbit serum	N	N	N

Code:

N—No specific fluorescence; ±—Equivocal green fluorescence; 1—Minimal green fluorescence; 2—Moderate green fluorescence; 3—Bright green fluorescence; 4—Brilliant green fluorescence.

laxis experiment are listed in Table V. The readings represent the maximum diameters of dye at the injection sites.

The small differences between reactions induced by malignant melanoma and those induced by human skin homogenates injected intravenously into guinea pigs with skin sites prepared with rabbit antimalignant melanoma serums were not considered significant.

DISCUSSION

An attempt was made to demonstrate specific antibodies to elements of human malignant melanoma tissue in the serums of rabbits in-

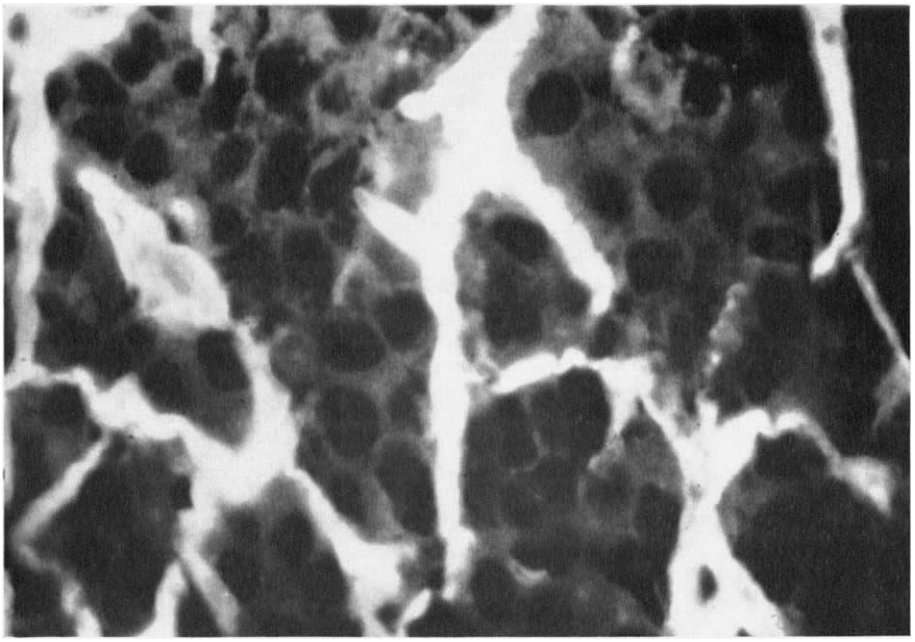
TABLE IV

Specific staining reactions of human skin

Serum	Papillary layer of dermis	Ground substance between collagen	Epidermis, collagen or elastic tissue
Antihuman malignant melanoma, antihuman skin, antihuman gamma globulin or antihuman albumin serums	3-4+	3-4+	N
Antiovalbumin	N	N	N
Antihuman malignant melanoma or antihuman skin serum absorbed with:			
1. Human spleen	N	N	N
2. Human plasma, beef liver or beef spleen	3-4+	3-4+	N
Antihuman albumin serum absorbed with:			
1. Human spleen or human plasma	N	N	N
2. Beef liver or beef spleen	2-3+	2-3+	N

Code:

N—No specific fluorescence.
1—Minimal green fluorescence.
2—Moderate green fluorescence.
3—Bright green fluorescence.
4—Brilliant green fluorescence.



Code:

FITC = Fluorescein Isothiocyanate
GARGG = Goat Anti-Rabbit Gamma Globulin

FIG. 1. Section of metastatic amelanotic malignant melanoma treated with post-immunization serum of a rabbit injected with human skin after which FITC conjugated GARGG was applied. (Magnification × 169.) *Shows:* Stroma of tumor brilliant green. Tumor cells show dull, non-specific green fluorescence.

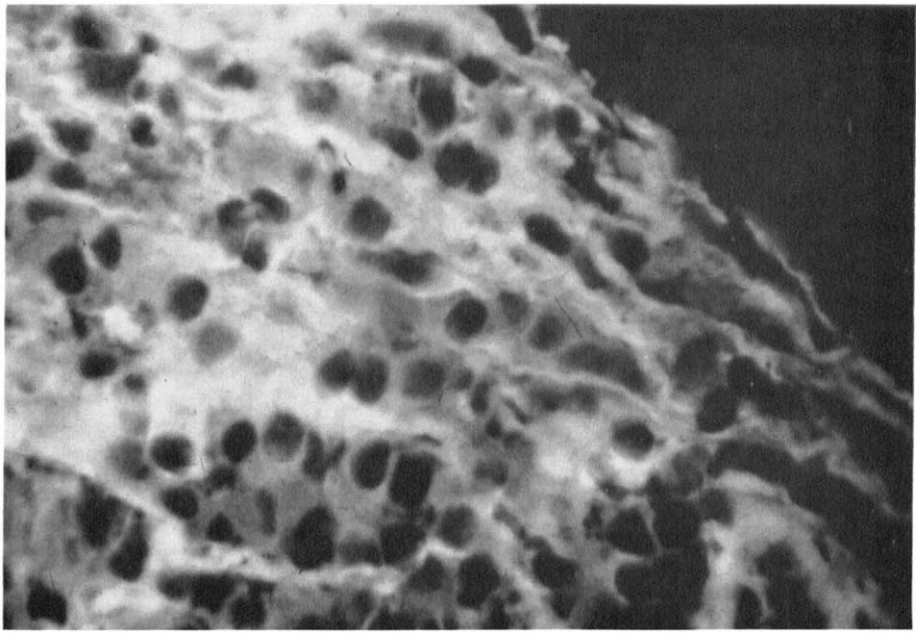


FIG. 2. Section of metastatic amelanotic malignant melanoma treated with the post-immunization serum of a rabbit injected with human malignant melanoma after which FITC conjugated GARGG was applied. (Magnification × 169.) *Shows:* Brilliant green fluorescence of stromal strands and cytoplasm of plasma and/or mononuclear cells. Tumor cells (upper right hand corner) are non-fluorescent.

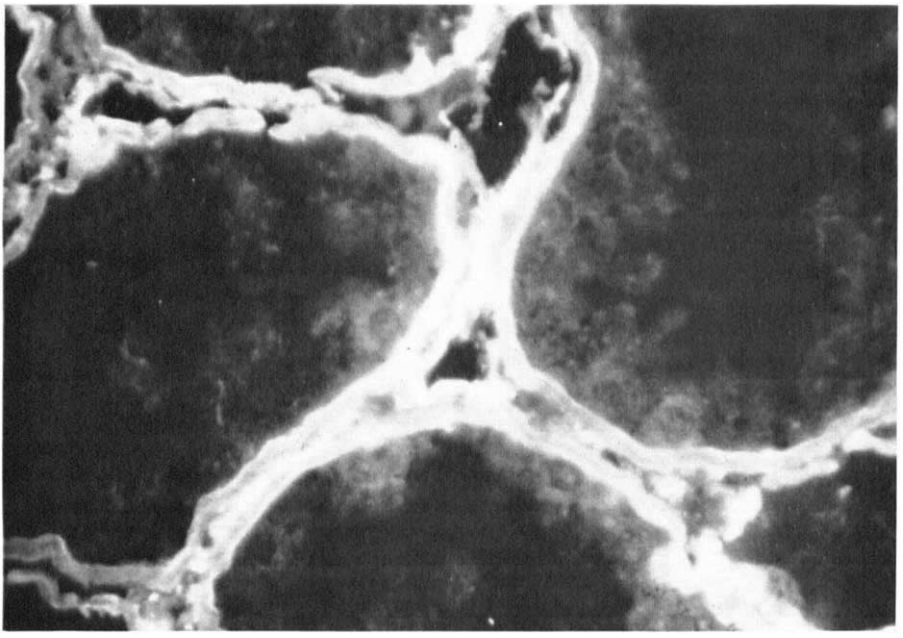


FIG. 3. Section of human kidney treated with post-immunization serum of a rabbit injected with human malignant melanoma after which FITC conjugated GARGG was applied. (Magnification × 169.) *Shows:* Brilliant green staining of stroma of renal tubules.

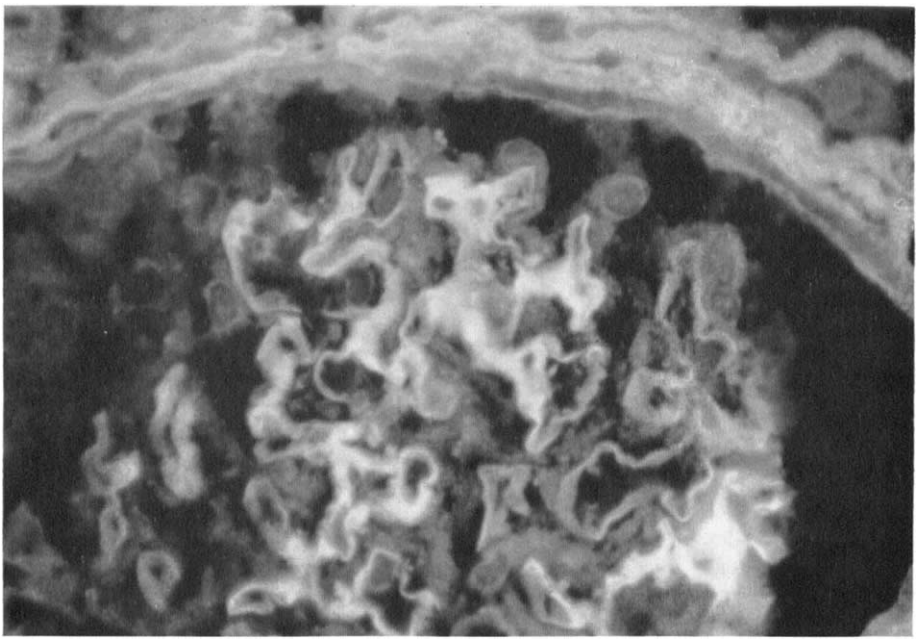


FIG. 4. Section of human kidney treated with post-immunization serum of a rabbit injected with human malignant melanoma after which FITC conjugated GARGG was applied. (Magnification × 169.) *Shows:* Brilliant green staining of glomerular basement membrane.

TABLE V

Passive cutaneous anaphylaxis

Total number	Serums injected intradermally	Number of rabbits in each group	Reactions in millimeters*	
	Type		Human malignant melanoma	Human skin
10	Antihuman malignant melanoma	1	21	19
		1	20	18
		1	19	10
		1	11	3
		6	0	0
1	Antihuman skin	1	0	0
2	Antihuman gamma globulin	1	16	5
		1	0	0
1	Antiovalbumin	1	0	0
1	Freund's adjuvant and normal saline alone	1	0	0
1	Normal rabbit serum	1	0	0

* Readings represent the average reading for 4 different guinea pigs in each experiment.

jected with homogenates of the tumor tissue. The following serum antibody reactions were found by the immunofluorescence technic:

A.—Fluorescein conjugated rabbit antimelanoma serum was found to stain the connective tissue stroma of tissue sections of human malignant melanoma. In order to determine if this reaction was tumor specific, sections of normal human skin and kidney were exposed to the same antiserum. In normal human skin, staining occurred in the papillary layer of the dermis, in the stroma between collagen bundles and in the stroma around adnexa. In human kidney, the stroma surrounding renal tubules and basement membrane of glomerular capillaries and Bowman's capsule specifically fluoresced. Rabbit antihuman skin serum reacted in a manner identical to the rabbit antimelanoma serum. It was possible to block the stromal staining of the conjugated antihuman malignant melanoma serum by prior incubation of the tissue sections with unconjugated rabbit antihuman skin serum. Absorption of the antimelanoma serum with normal human spleen, lung, or kidney removed the stromal staining. Using the indirect Coon's technic, rabbit antihuman albumin and antihuman gamma globulin showed the same pattern of staining as rabbit antihuman skin and antihuman melanoma serum; however, the staining was of less intensity. Contrary to the finding with antihuman skin and antihuman melanoma serums, when the antihuman albumin and antigamma globulin serums were directly conjugated with fluorescein isothiocyanate, this staining was lost. This may be due to the fact that a component of rabbit serums other than 7S gamma globulin was responsible for the staining and that this component was detected using conjugated goat antirabbit gamma globulin. The goat antirabbit gamma globulin used was shown by immunoelectrophoretic analysis to possess antibodies to components of rabbit serum other than 7S gamma globulin. It is also possible that the factor was diluted out during the conjugation procedure. Milazzo (15) demonstrated by precipitin tests that antiserum to human globulin reacted with human glomerular antigen. Tan and Kaplan (16) found that antiserum to a fraction of mouse serum beta-globulin was found reactive by the immunofluorescence technique with basement membrane of renal glomeruli and tubules and with basement membrane and connective tissue in other organs.

The above findings reveal that the staining of stroma was not tumor specific; they suggest an immunologic relationship of cutaneous, renal and malignant melanoma stromal antigens.

B.—The walls of blood vessels stained in tissue sections of human kidney, human skin and human malignant melanoma when these were incubated with either rabbit antihuman melanoma, antihuman skin, antihuman gamma globulin, or antihuman albumin serums. This staining was best absorbed out by human brain or spleen powder. The exact site of staining or type of vessels stained could not be precisely determined. Cruickshank and Hill described similar staining of blood vessel walls with antiserums prepared against renal connective tissue (17, 18).

C.—Conjugated rabbit antihuman malignant melanoma serum stained the cytoplasm of plasma cells and/or large mononuclear cells within the tumor tissue. These cells were also stained by conjugated rabbit antihuman skin and rabbit antihuman gamma globulin serum. On immunoelectrophoresis all these serums were shown to possess an antihuman gamma globulin antibody. Absorption of the antiserums with either human gamma globulin, plasma, spleen, liver, kidney, lung, brain, or blood cells removed the staining. They were not stained by conjugated rabbit antihuman albumin or antiovalbumin serum. Evidently the staining was due to the presence of gamma globulin. While significant amounts of gamma globulin were not detectable in or on the tumor cells, 7 out of 13 human malignant melanomas showed the presence of gamma globulin in cells believed to be plasma cells and/or large mononuclear cells. While the gamma globulin in these cells may have been sufficient to act as the antigen, other sources within the tissues used for immunization of the rabbits must be considered. Since rather intense immunization schedules were used, small amounts of gamma globulin in the tissue spaces or intravascularly may have provoked the response. The fact that normal human skin injected into rabbits resulted in the production of antibodies to human gamma globulin would favor the non-tumor specificity of this finding.

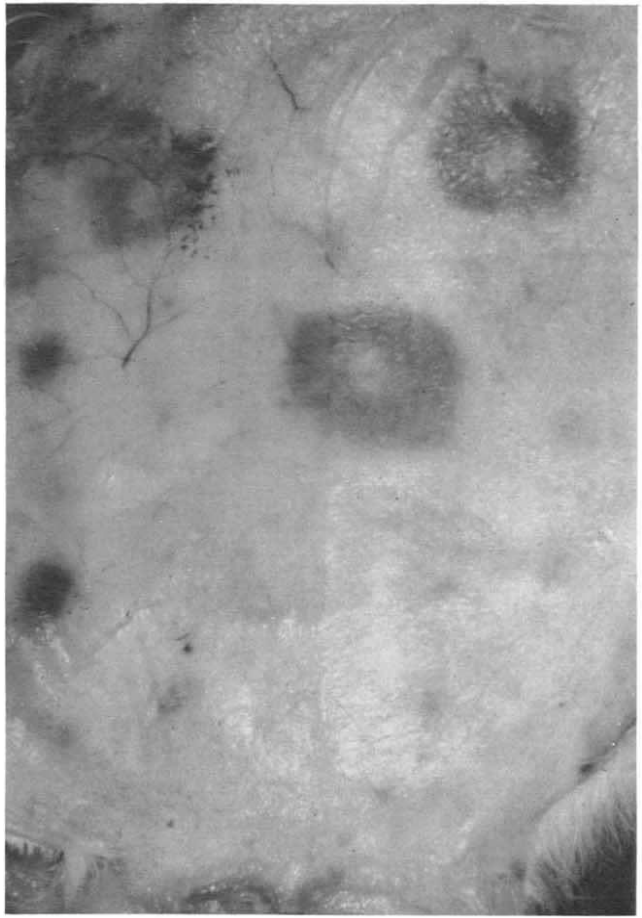


FIG. 5.

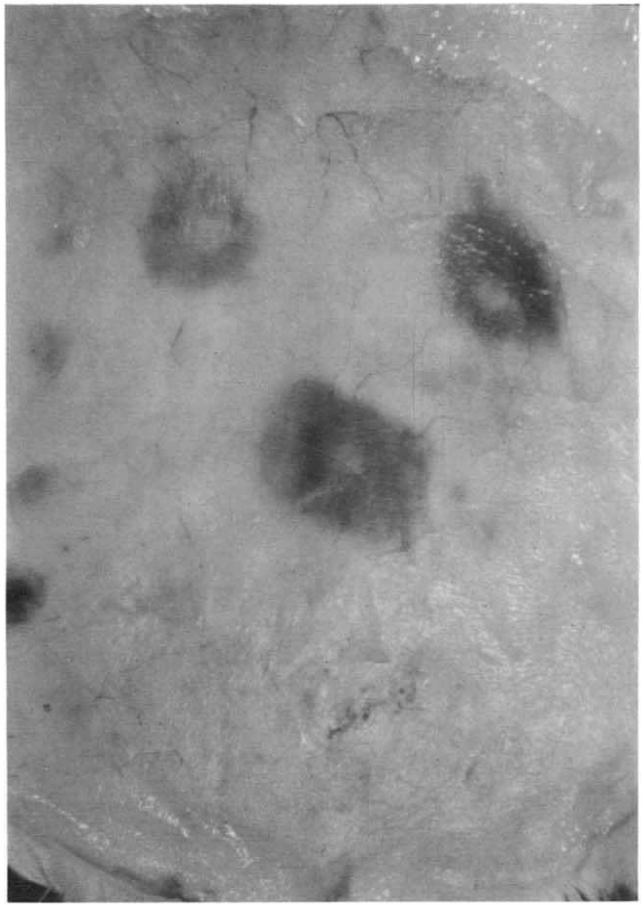


FIG. 6.

FIG. 5. Results of passive cutaneous anaphylaxis in guinea pigs. Reactions at sites prepared with rabbit anti-human malignant melanoma serum injected intradermally followed by the intravenous administration of human skin homogenate plus Evans blue. Compare to Figure 6.

FIG. 6. Results of passive cutaneous anaphylaxis in guinea pigs. Reactions at sites prepared with rabbit anti-human malignant melanoma serum injected intradermally followed by the intravenous administration of human malignant melanoma homogenate plus Evans blue. Compare to Figure 5.

D.—Neither conjugated rabbit antihuman malignant melanoma serum nor the other antiserums studied significantly stained the cytoplasm of malignant melanoma tumor cells.

Virtually no information is available concerning the threshold sensitivity of the immunofluorescence technic. Passive cutaneous anaphylaxis, however, has been demonstrated to be a sensitive procedure for the demonstration of antigen-antibody reactions. With this method no differences could be demonstrated when either malignant melanoma or skin emulsions were injected intravenously into guinea pigs with skin sites prepared with rabbit antimalignant melanoma serums (Figs. 5 and 6). Thus, this independent immunologic tool also failed to demonstrate the presence of tumor-specific antigen in human malignant melanoma.

The reasons why in this study attempts to obtain antibody directed toward tumor cells themselves failed is unknown; however, the following possibilities were considered:

1. Whole tumor homogenate has many potent antigens and the rabbit may not have been able to produce a detectable circulating antibody response to weaker antigens. Therefore, if tumor specific antigens exist and are weak antigens, the animals would fail to respond to them.
2. The technics employed are incapable of detecting any cell-bound antibody which the rabbits might have produced.
3. The rabbit may not be the laboratory animal of choice for attempts to immunize with tumor tissue (19).
4. Malignant melanoma may indeed, not possess a specific tumor antigen.

SUMMARY

1. Rabbits immunized with tissue from human malignant melanoma, human skin and other control materials, developed serum antibodies which were shown by the immunofluorescence technic to react with: (a) stroma of malignant melanoma tissue; (b) cytoplasm of plasma cells and/or large mononuclear cells within the tumor; (c) stroma surrounding tubules in human kidney; (d) basement membrane of the renal glomeruli; (e) walls of some blood vessels; (f) pars papillaris of human skin and (g) scattered areas between dermal collagen bundles.

2. The staining of the cytoplasm of plasma cells and/or large mononuclear cells in 7 out of 13 human malignant melanomas studied was due to the presence of gamma globulin. The significance of this finding is unknown.

3. Attempts to demonstrate rabbit antibodies specific for human malignant melanoma cells were unsuccessful.

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